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14. ABSTRACT
Amnion Derived Multipotent Progenitor Cells (AMPCs) and their secreted amnion-derived cellular cytokine suspension (ACCS) may have the potential to enhance wound healing and tissue repair. The objective of this proposal is to test the ability of AMPCs and ACCS to effect recovery after spinal cord injury (SCI), according to two hypothesis-driven goals: A) Does acute (2 day delay) AMP cell transplantation after SCI improve functional locomotor recovery and histological injury measures?; and B: Is ACCS sufficient to promote recovery of function after SCI, or synergistic when administered in combination with AMP cells? AMPCs and ACCS will be transplanted into a well-established model of contusion induced SCI, contusion injury being the most common and clinically relevant form of damage in the human clinical population, according to the experimental parameters of the hypotheses outlined under the objectives and in the detailed experimental design. Regaining partial function can lead to improved functional mobility and sensation, improving quality of life and reducing lifetime costs associated with SCI.

15. SUBJECT TERMS
None listed.

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INTRODUCTION:

Stemnion Inc. is developing Amnion Derived Multipotent Progenitor Cells (AMPCs) and their secreted amnion-derived cellular cytokine suspension (ACCS) as a platform technology to enhance wound healing and tissue repair. AMPCs display many of the promising features of stem cells, including the ability to differentiate into varied cell types. However, since they are isolated from the membranes of full-term placentas, they are not subject to the ethical, social, or religious strictures that impact embryonic stem cell research. AMPCs are readily available, abundant, and have been shown to proliferate in culture. In addition, AMPCs do not express telomerase, reducing the possibility of tumorigenesis upon transplantation. Stemnion's preliminary research has suggested that AMPCs are non-immunogenic and have extraordinary potential in the wound healing and tissue repair arena, since their unique secretory profile (ACCS) contains many of the essential cytokines and growth factors involved in wound healing. Accordingly, pilot experiments have established proof-of-concept for AMPCs and ACCS to enhance wound healing and tissue repair in both traumatic and elective wounds including burns, contaminated open wounds, surgical abdominal incisions, and traumatic brain injuries (TBI).

The vast majority of patients with traumatic SCI exhibit histopathology that is the result of a partial, not a complete, injury. In other words, while paralysis may be complete, intact spinal cord tissue remains, which suggests the opportunity for improving functional recovery either via limiting the initial damage or supporting enhanced wound healing post-trauma. The long-term paralysis associated with SCI results from a complex set of events, including, but not limited to: inflammation, a spreading area of necrotic and apoptotic cell death of neurons and myelinating oligodendrocytes, axonal loss and demyelination, as well as astroglial scarring of the injured area and the generation of myelin and chondroitin sulphate proteoglycan (CSPG) associated inhibitors of regeneration. Local application of AMPCs, ACCS, or the combination of the two may limit the initial degree and spread of injury, and thus improve the degree of recovery. Moreover, AMPCs secrete proteins (ACCS) that have been shown to mediate cellular processes of tissue repair, opening up the possibility that increasing the healing trajectory with AMP transplantation could decrease long-term glial scarring or inhibitory matrix generation. Additionally, data from other work at Stemnion suggests that AMPCs and/or ACCS could work directly to down-regulate fibroblast and glial cell recruitment and activity, further limiting excessive scar formation.

Recent advances using embryonic and adult stem cells have shown promise in treatment of paralysis. Using standard locomotor tests, improvement in function has been shown experimentally with autologous bone marrow cells (Sykova et al., 2006), amniotic epithelial cells (Wu et al., 2006), umbilical cord blood cells (Nishio et al., 2006), and mesenchymal stem cells (Cizkova et al., 2006), human oliogoprogenitor cells (Keirstead et al., 2005), and human neural stem cells (Cummings et al., 2005). A major problem with clinical transplantation and translation of most stem cells is immunogenicity and associated engraftment failure or rejection. Because Stemnion's AMPCs has been suggested to be non-immunogenic and survive xenograft transplantation, as well as exhibit immunomodulatory properties, this cell population may be somewhat uniquely suited as an early cell transplantation therapy targeting acute reduction of damage rather than subacute or chronic replacement of cells.

BODY:

The originally approved statement of work outlined two Aims: A) Does acute (2 day delay) AMPC transplantation after SCI in NOD-scid mice improve functional locomotor recovery and histological injury measures? And, B: Is ACCS sufficient to promote recovery of function after SCI, or synergistic when administered in combination with AMPCs in NOD-scid mice? In addition, we proposed to complete histology from preliminary data on 9 day delay AMPC transplants in NOD-scid mice, and applied for and received approval to conduct an additional comparison experiment testing 2 day and 9 day delay transplantation in a parallel experiment.

The proposed and approved experiments can be summarized as follows:

1) Complete histology for AMPC survival and lesion volume for previously injured and transplanted 9 day delay AMPC transplanted NOD-scid mice in which behavioral analysis had already been completed and presented as preliminary data.
2) Transplant AMPCs 2 days post-SCI, comparing epicenter versus rostral-caudal parenchymal transplants. Endpoints: behavioral testing, histology for AMPC survival and ex vivo MRI lesion volume.
3) Transplant AMPCs 9 days and 2 days post-SCI, epicenter transplants only, comparing transplantation timepoint within a single experiment. Endpoints: behavioral testing, histology for AMPC survival.
4) Compare ACCS versus AMPC administration at the optimal timepoint and transplantation time determined from the above experiments. Endpoints: behavioral testing, histology for AMPC survival, lesion volume.

The progress and results for each of these will be summarized below. All data graphs/figures are included under the Appendix. The Appendix is organized by Project sections that refer to internal project designations that are indicated for each experiment below.

1) Complete histology for AMPC survival and lesion volume and ex vivo MRI for previously injured and transplanted 9 day delay AMPC transplanted NOD-scid mice in which behavioral analysis had already been completed and presented as preliminary data. Internally designated Project 51.1

Analysis for this project was completed under the DOD contract as proposed. The project was designed to test the effect of AMPCs on locomotor recovery after contusion SCI with transplantation into immunodeficient NOD-SCID mice. All non-surgical and surgical procedures and monitoring / post-operative care procedures were described in detail under Sections 5 and 6 and approved by IACUC review. A moderate T9 contusion injury (50kd) was produced using the IH Impactor. The study had four groups: rostral-caudal transplant, rostral-caudal control, epicenter transplant and epicenter control. Transplants of 75,000 cells were made nine days post-injury (sub-acute) and animals were sacrificed at three months post-graft. BBB/BMS, Catwalk and LadderBeam were used for behavioral assessment. Staining for GFAP was used for histological evaluation of cord volume parameters (total cord, GFAP, lesion and spared). Graft survival was evaluated using human marker immunocytochemistry (ICC). Staining to test for cell engraftment can included a panel of three human nuclei antibodies routinely used in the Anderson laboratory for other human cells. Project 51.1 open field analysis showed a trend for improved recovery following injection of hAMPs into the injury epicenter, but was not statistically significant by repeated measures ANOVA (51.1 Figure 1 Behavior.jpg). However, this behavioral trend was supported by more sensitive tests, as LadderBeam analysis of stepping errors indicated a significant improvement in the task following hAMPs treatment (51.1 Appendix Figure 1 Behavior.jpg). This was further supported by CatWalk Gait analysis, which indicated a significant improvement in paw angle for the hindlimbs and print length for the right forepaw (51.1 Appendix Figure 2 Behavior.jpg). Preliminary histology analysis of 24 animals revealed no difference between groups for GFAP, lesion, or spared tissue volumes (51.1 Appendix Figure 3 Histology.jpg). Immunostaining showed no surviving cells at three months post-graft (data not shown, see Project 51.2 data (below) for an example).

Status: Complete.

2) Transplant AMPCs 2 days post-SCI, comparing epicenter versus rostral-caudal parenchymal transplants. Endpoints: behavioral testing, histology for AMPC survival and ex vivo MRI lesion volume. Internally designated Project 51.2

Based on the behavioral data from the initial 9 day delay experiment, it appears that there may be a wound healing effect in the animals receiving epicenter AMPC transplantation, which could be a quite interesting finding. The logical prediction is that an effect on wound healing would be enhanced at earlier timepoints after SCI, when there is greater opportunity to modify secondary injury processes. Accordingly, the effect of a more acute transplantation time on locomotor outcome was proposed for investigation. A 2 day delay was selected based on the clinical relevance of surgical intervention within the first 48 hours post-SCI. All non-surgical and surgical procedures and monitoring / post-operative care procedures were described in detail under Sections 5 and 6 and approved by ACURO and IACUC review. A moderate T9 contusion injury (50kd) was produced using the IH Impactor. 2 days post-SCI, human ADCs were injected via a pulled glass micropipette attached to a nanoinjector either into four sites around the injury center (2 rostral and 2 caudal, right and left of the center) or as a single injection directly into the injury epicenter. 250nl of cells were injected for each rostral caudal site for a total of 1 ul for each animal. 2ul of cells were injected for the epicenter site. Cells were be injected at a concentration of 75,000 cells per µl sterile HBSS. Vehicle will consist of sterile HBSS.

No significant differences were detected between groups using repeated measures ANOVA followed by post-hoc Bonferroni-Dunn tests in the main BMS score (51.2 Appendix, Figure 1a). However, a trend for parenchymal transplant animals to be worse than epicenter controls at the end of the experiment (28d survival) is clearly apparent in the BMS subscore (51.2 Appendix, Figure 1b). In contrast to the behavioral data, MRI analysis of lesion volume revealed a surprising reduction in lesion volume in the parenchymal AMP transplant group (AMP-RC) in comparison with both vehicle control groups and the epicenter AMP transplant group (AMP=Epi). ANOVA $p<0.05$, $F=3.406$ (51.2 Appendix, Figure 2a). * Indicates $p<0.05$ versus all other groups. However, immunocytochemistry to identify engrafted cells using labeling for human nuclei in sections from the engrafted animals has not revealed evidence for surviving cells 28d post-transplant (51.2 Appendix, Figure 2b).

Status: Analysis of supplemental behavioral tasks (CatWalk and LadderBeam) is being completed. Project will be complete after this analysis.

3) Transplant AMPCs 9 days and 2 days post-SCI, epicenter transplants only, comparing transplantation timepoint within a single experiment. Endpoints: behavioral testing, histology for AMPC survival. Internally designated Project 51.3

As described above, in P51.1 we tested both epicenter and rostral-caudal parenchymal transplant locations, and only the epicenter transplants improved locomotor recovery. All experiments were conducted in a blinded fashion, and none of the technicians involved in behavioral testing had any access to coded animal data identifying the experimental groups. Because of difference between the epicenter and parenchymal transplant groups and the magnitude of the

behavioral change observed, we believe it is unlikely that there is a false positive for recovery of function in that experiment.

As also described above behavioral analysis for P51.2 did not reveal improvements in locomotor recovery similar to those observed in the case of delayed transplantation (9 days post-SCI). There is a concern that two factors may have contributed to this result: 1) the cell lines shipped to us by Stemnion for the initial project (P51.1) and current project (P51.2) were not the same; 2) the culture medium used in cell preparation for the initial project (P51.1) and current project (P51.2) were not the same. This is because pursuant to preparing for clinical translation, Stemnion has instituted a change in culture techniques that excludes reagent containing/exposed to animal products from the culture medium.

Based on the results above and in consultation with Stemnion investigators and Dr. Curley, we therefore redesigned the scope of work to incorporate a parallel analysis transplantation timing directly comparing 9dpi with 2dpi and focusing specifically on the epicenter group, this project was designated P51.3.

No significant differences in locomotor recovery were observed in P51.3 (51.3 Appendix, Figure 1), as w as the case in P51.2. Further, preliminary histological analysis has shown little evidence for AMPC engraftment using immunostaining for human nuclei, as was the case for the P51.2 2d post-SCI transplantation paradigm (data not shown).
Status: Additional immunocytochemical analysis for AMPC engraftment in progress, project will be complete after this analysis.

4) Compare ACCS versus AMPC administration at the optimal timepoint and transplantation time determined from the above experiments. Endpoints: behavioral testing, histology for AMPC survival, lesion volume.

We feel that at this point it is appropriate to proceed with Aim B of the contract at this juncture, focusing on feasibility of ACCS in SCI, and complete these studies in a model that has optimal clinical relevance for wound healing (Sprague Dawley rat) rather than cell engraftment (NOD-scid mouse). Based on discussions with Dr. Curley, the appropriate IACUC modifications have been submitted at UCI, and when approved will be submitted through ACURO while final histological engraftment analyses are ongoing from P51.3. A more detailed rationale for this shift is included under CONCLUSIONS below.
Status: Replaced with a test of ACCS administration via intrathecal catheter, see 'Aim B Alternative Proposed Project Plan for testing ACCS' following CONCLUSIONS below.

KEY ACCOMPLISHMENTS:
Noted above

REPORTABLE OUTCOMES:
No publications at this time.

CONCLUSIONS:

Our working hypothesis is that the promising preliminary data reflect transient cell survival and synthesis of trophic or other factors that may alter the injured/wound healing environment. Additionally, variation in AMPC cell lot effects could suggest that cell engraftment/survival severely compromised in the injured spinal niche, even in the xenograft friendly NOD-scid mouse. The success we have historically had with engraftment of other CNS cell types in this model points to a niche effect in this case as the basis for survival failure. Full analysis of this issue would require a timecourse study that is outside the scope of this project.

As these studies have been in progress Stemnion has made significant advances with the ACCS side of their research program, with several key advances that pertain directly to this SCI project. First, they have reported that multiple treatments with ACCS is as as or more effective than AMPCs in wound healing paradigms (data submitted as a part of Stemnion IND application, graph appended below). Second, they have recently (December 2008) received FDA approval of their IND application for use of ACCS in a Phase I trial in burn patients. Accordingly, in terms of project planning and regulatory issues, use of a cell-derived media product may be a more rapid route to trial than a product that is a cell-based therapeutic.

Accordingly, we feel that at this point it is appropriate to proceed with Aim B of the contract at this juncture, focusing on feasibility of ACCS in SCI, and complete these studies in a model that has optimal clinical relevance for wound healing (Sprague Dawley rat) rather than cell engraftment (NOD-scid mouse). Based on discussions with Dr. Curley, the appropriate IACUC modification has been submitted at UCI, and when approved will be submitted through ACURO while final histological engraftment analyses are ongoing from P51.3. The proposed project plan for ACCS is below:

Aim B Alternative Proposed Project Plan for testing ACCS:

Amnion-derived Cellular Cytokine Suspension (ACCS) is a suspension of physiologic levels of growth factors and tissue inhibitors of metalloproteinases derived from Amnion Derived Multipotent Progenitor Cells (AMPCs). Based on wound healing data with ACCS in skin injury models (Steed, DL, et al., 2008 and Franz, MG., et al., 2008), we hypothesized that ACCS administration would minimize lesion volume and glial scaring post-SCI, promoting the capacity for regenerative responses.

All of the originally proposed studies using AMPCs and ACCS made use of NOD-scid mice, a constitutively immunodeficient mouse model in which there is a minimal xenograft rejection response, in order to maximize AMPC transplant survival. A principal overall goal of these studies was to investigate and compare the efficacy of AMPC and ACCS administration. However, analysis of the results from the previously approved AMPC transplantation studies has revealed that AMPCs do not survive well in the injured spinal cord. These data are in striking contrast to previous transplantation work with other stem cell and progenitor populations in the Anderson Lab and CDRF Core, which have shown robust survival and engraftment in the NOD-scid SCI model.

A significant limitation of the NOD-scid mouse SCI model with regard to wound healing is the lack of cavitation in all mouse SCI models, which is a normal clinical feature of human SCI, and the altered immunological response of these animals. Recent data have suggested that ACCS may exert some activity by altering the immunological response to injury, an effect that could be partially abrogated in the NOD-scid model. Additionally, our collaborators have recently (December 2008) received FDA approval of their IND application for use of ACCS in a Phase I trial in burn patients. Further, a second body of recent evidence published since the approval of this protocol has suggested that multiple administrations of ACCS are more efficacious in promoting wound healing that either AMPC transplant, or single ACCS treatment alone (Franz, MG., et al., 2008). Finally, in terms of project planning and regulatory issues, use of a cell-derived media product may be a viable potential SCI therapeutic than a product that is a cell-based. Accordingly, given the lack of successful engraftment of AMPCs in the NOD-scid SCI model, and the potential for improved efficacy for ACCS in an immunocompetant rat SCI model, we propose to alter the original final experiment in this trio (section 3.3.2 experiment C) to focus on a moderate contusion injury in Sprague Dawley rats.

Change of route of administration:

In the original version, the effect of ACCS administration was tested using epicenter injection via a pulled glass micropipette attached to a nanoinjector. As noted above, recent data has suggested that multiple administrations of ACCS are more efficacious in promoting wound healing that either AMPC transplant, or single ACCS treatment alone (Franz, MG., et al., 2008). Accordingly, we propose to alter the route of administration to deliver ACCS via intrathecal pump, which will provide continuous delivery of ACCS for 14d post-SCI. This is an already approved procedure under the existing protocol.

Group Tr	eatment	Strain/Species USDA		Code	N
1 Vehicle		Sprague-Dawley rats	D	ADULT	20
2 ACCS		Sprague-Dawley rats	D	ADULT	20
			AD	ULT Rats	40
			AD	ULT-S Rats	0
			TOT	AL N	40

All non-surgical and surgical procedures and monitoring / post-operative care procedures are described in detail under Sections 5 and 6. All procedures will be performed under isoflurane anesthesia. The spinal cord will be exposed at T9 and a 200kd contusion injury produced using the IH Impactor. Immediately following SCI, a fine intrathecal catheter, attached to an osmotic minipump, will be inserted at L2 and gently threaded to T10. The osmotic pumps will be implanted subcutaneously and contain either ACCS or vehicle. Animals will be anesthetized 2 weeks post-injury and pumps will be removed. The catheter placement, pump placement, and pump removal have been previously approved by IACUC and are detailed in 4.7 and 4.13 of section 6 of the application. For all procedures, after suturing, all animals will be monitored until they recover from anesthetic, and maintained on water-jacketed heating pads at 37° C overnight. Animals will be group housed in cages with Alpha-dri bedding, monitored a minimum of 2x/day for a minimum of 2 weeks post-SCI for signs of debilitation or skin lesions, and their bladders expressed a minimum of 2x/day by manual crede during this period. Animals will be monitored 1x/day thereafter until sacrifice. Animals will be tested on BBB (Pre-injury, 2 days post-SCI, 1 week post-SCI, and each week for 4 weeks), gait analysis (pre-SCI and 4 weeks post-SCI), and ladderbeam (pre-SCI and 4 weeks post-SCI). Animals will be euthanized and tissue harvested for provision 4 weeks post-SCI.

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Cummings B. J., Engesser-Cesar C., Cadena G., and Anderson A. J. A new method for quantitative assessment of locomotor function after spinal cord injury in the mouse: validation of a horizontal ladder beam task across strain and injury severity. 2007. Behavioral Brain Research. 177(2):232-41

Cummings BJ, Uchida N, Tamaki SJ, Salazar DL, Hooshmand M, Summers R, Gage FH, Anderson AJ. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci U S A. 2005 Sep 27;102(39):14069-74. Epub 2005 Sep 19.

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Kilot M, Lustgarten JH: Strategies to promote regeneration and recovery in the injured spinal cord. Neurosurg Clin N Amer 1990; 1: 757-759.

Nishi R. A., Liu H., Cadena G., Thamkruphat T., Chu Y., Hamamura M. J., Su M-Y., Nalcioglu O., and Anderson A. J. Characterization of graded contusion spinal cord injury in C57 Bl/6 mice and comparison of lesion volume determination using immunocytochemistry and Ex Vivo MRI. 2007. Journal of Neurotrauma. 24(4):674-89.

Nishio Y, Koda M, Kamada T, et al: The use of hemopoietic stem cells derived from human umbilical cord blood to promote restoration of spinal cord tissue and recovery of hindlimb function in adult rats. Neurosurg Spine 2006; 5: 424-432.

Sykova E, Homola A, Mazanec R, et al: Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. Cell Transplant 2006; 15: 675-687.

Wu ZY, Hui GZ, Lu Y, et al: Transplantation of human amniotic epithelial cells improves hindlimb function in rats with spinal cord injury. China Med J 2006; 119: 2101-2107.

APENDICIES:

1) Complete histology for AMPC survival and lesion volume and ex vivo MRI for previously injured and transplanted 9 day delay AMPC transplanted NOD-scid mice in which behavioral analysis had already been completed and presented as preliminary data. <u>Internally designated Project 51.1</u>

Figure 1a. 51.1 BBB and BMS Open Field Analysis

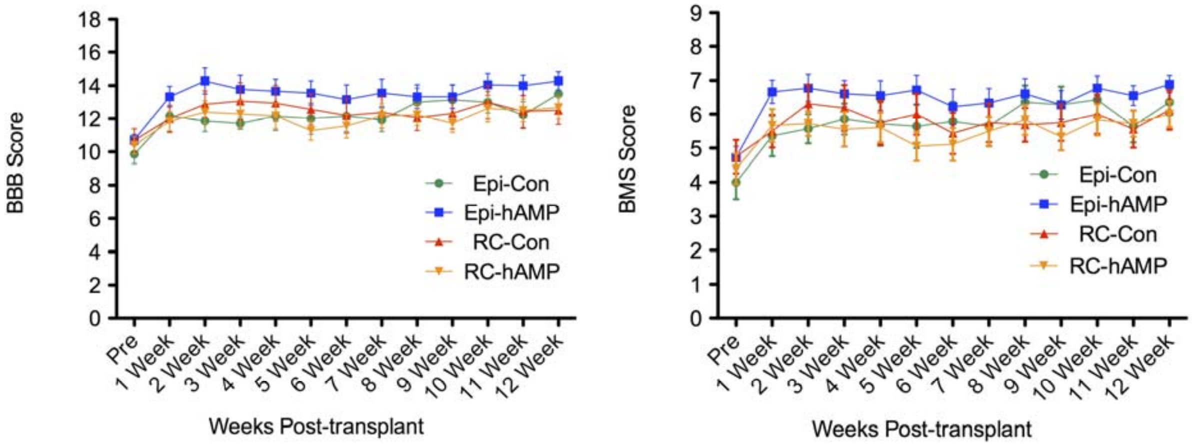
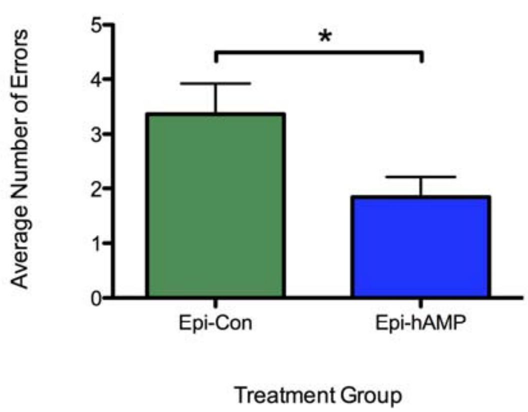


Figure 1b. 51.1 Ladderbeam Task



* Two-tailed t-test, $p<0.05$

Figure 1. Open Field Locomotor and Horizonal Ladder Beam Assessments for Project 51.1. A) BBB and BMS scores for experimental groups. While a strong trend was observed, no statistically significant differences were found between groups in repeated measures ANOVA analysis with post-hoc Bonferoni Dunn correction. B) Supplemental behavioral analysis of horizontal ladder beam testing focusing on the epicenter transplantation groups revealed a significant reduction in the number of hindlimb errors in animals that received hAMP transplants versus vehicle controls.

APENDICIES:

1) Complete histology for AMPC survival and lesion volume and ex vivo MRI for previously injured and transplanted 9 day delay AMPC transplanted NOD-scid mice in which behavioral analysis had already been completed and presented as preliminary data. <u>Internally designated Project 51.1</u>

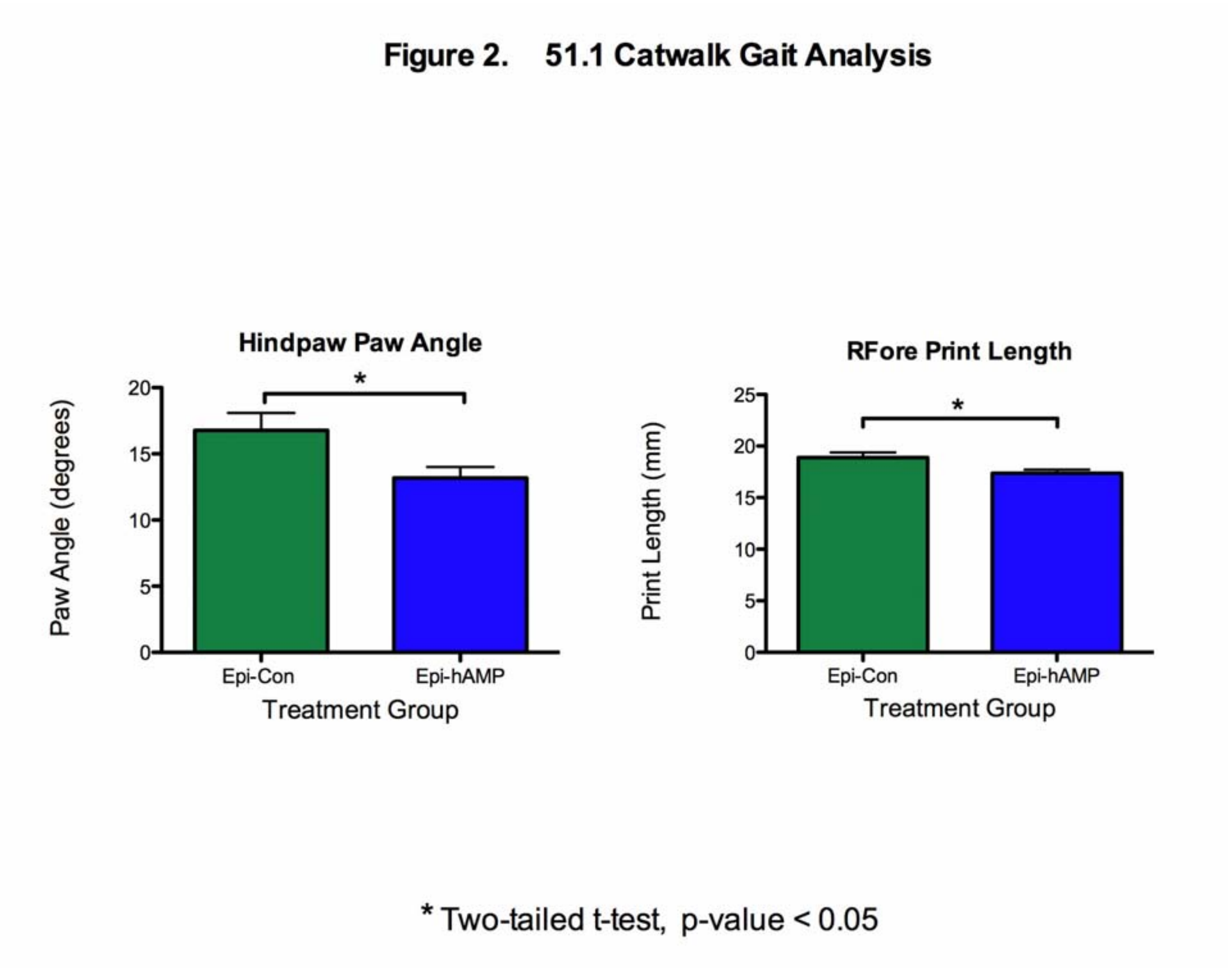


Figure 2. CatWalk Kinematic Gait Analysis for Project 51.1. Supplemental behavioral analysis of using CatWalk Gait testing focusing on the epicenter transplantation groups revealed a significant improvements in both hindlimb rotational paw angle and foreprint length in animals that received hAMP transplants versus vehicle controls.

APENDICIES:

1) Complete histology for AMPC survival and lesion volume and ex vivo MRI for previously injured and transplanted 9 day delay AMPC transplanted NOD-scid mice in which behavioral analysis had already been completed and presented as preliminary data. <u>Internally designated Project 51.1</u>

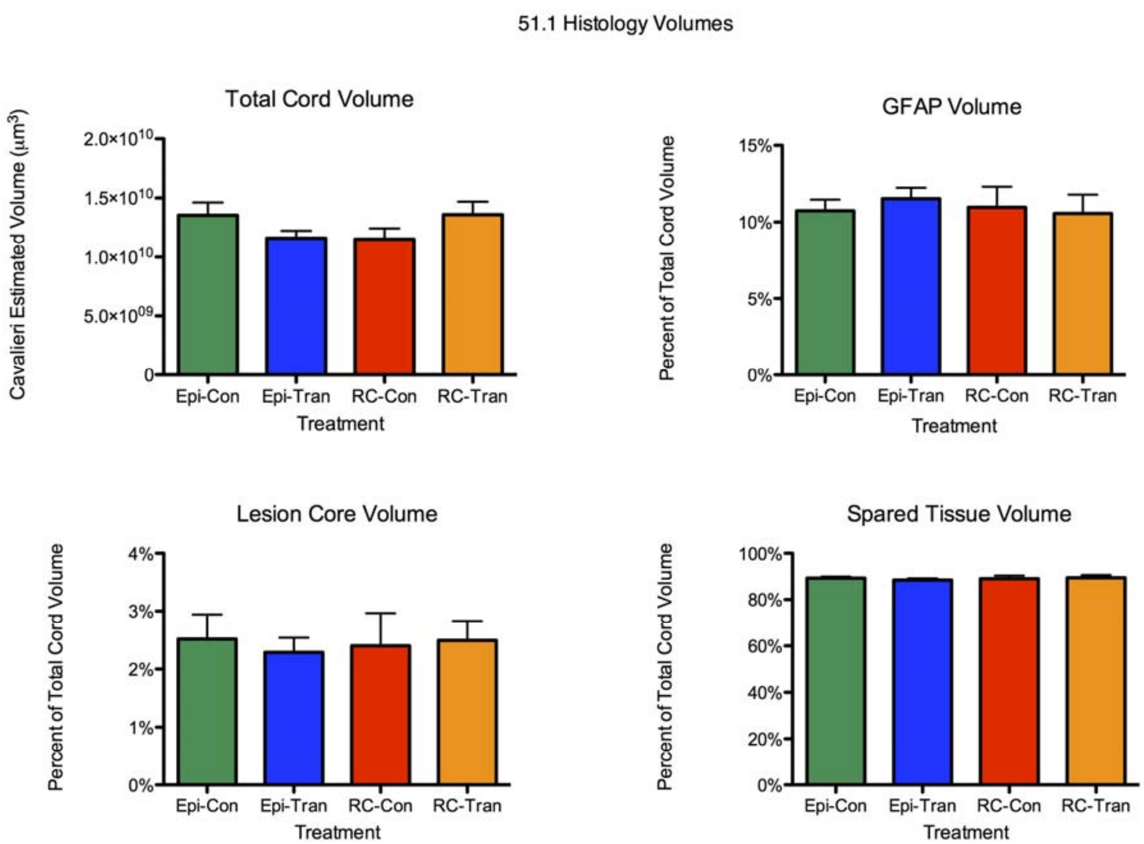
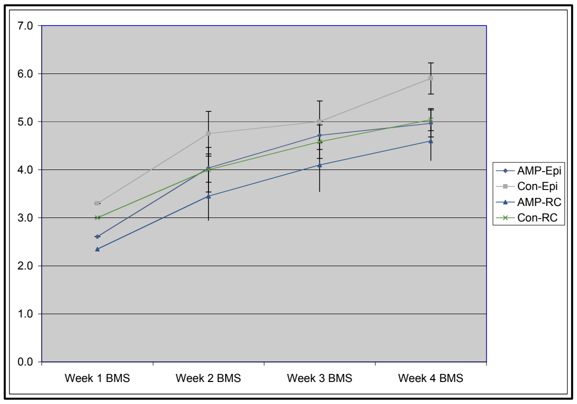


Figure 3. Unbiased Stereological Analysis of Lesion Parameters for Project 51.1. No statistically significant differences between groups were detected using Cavalieri sampling to determine total spinal cord volume (A), GFAP scar volume (B), lesion volume (C), or spared tissue volume (D). ANOVA followed by post-hoc t-test.

APENDICIES:

2) Transplant AMPCs 2 days post-SCI, comparing epicenter versus rostral-caudal parenchymal transplants. Endpoints: behavioral testing, histology for AMPC survival and ex vivo MRI lesion volume. <u>Internally designated Project 51.2</u>

A



B

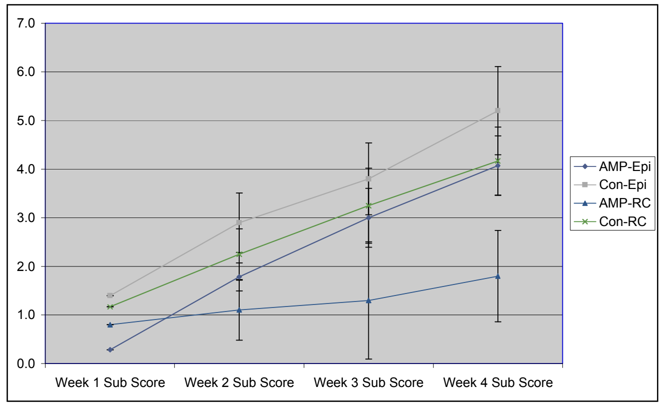
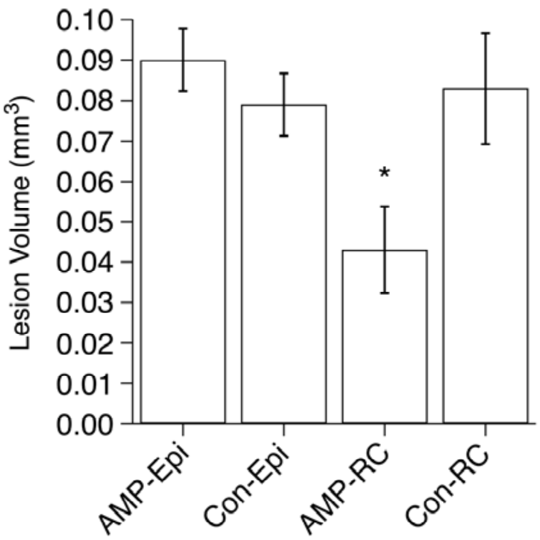


Figure 1. A) No significant differences were detected between groups using repeated measures ANOVA followed by post-hoc Bonferroni-Dunn tests in the main BMS score. B) BMS subscore analysis.

APENDICIES:

2) Transplant AMPCs 2 days post-SCI, comparing epicenter versus rostral-caudal parenchymal transplants. Endpoints: behavioral testing, histology for AMPC survival and ex vivo MRI lesion volume. **<u>Internally designated Project 51.2</u>**

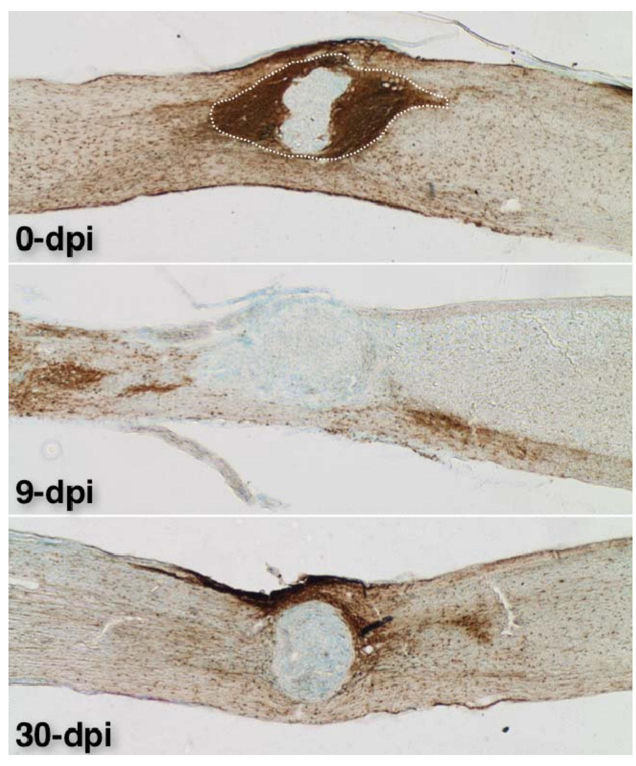
A



B

Figure 2. A) MRI analysis of lesion volume revealed a surprising reduction in lesion volume in the parenchymal AMP transplant group (AMP-RC) in comparison with both vehicle control groups and the epicenter AMP transplant group (AMP=Epi). ANOVA $p<0.05$, $F=3.406$ (graft at left, below). * Indicates $p<0.05$ versus all other groups. B) Immunocytochemistry to identify engrafted cells using labeling for human nuclei was conducted; no evidence of surviving cell engraftment is apparent. This animal represents the only transplanted case in which any labeling was observed. An example of immunolabeling for this marker after transplantation of human neural stem cells is appended below for comparison:

Cummings B.J., Hooshmand M. J., Salazar D. S., and **Anderson A. J.** Human neural stem cell mediated repair of the contused spinal cord: Timing the microenvironment. In: From Development to Degeneration and Regeneration of the Nervous System. Edited by Charles E. Ribak, Carlos Arámburo de la Hoz, Edward G. Jones, Jorge A. Larriva Sahd, and Larry W. Swanson. Oxford Press. 2008.



APENDICIES:

3) Transplant AMPCs 9 days and 2 days post-SCI, epicenter transplants only, comparing transplantation timepoint within a single experiment. Endpoints: behavioral testing, histology for AMPC survival. <u>Internally designated Project 51.3</u>

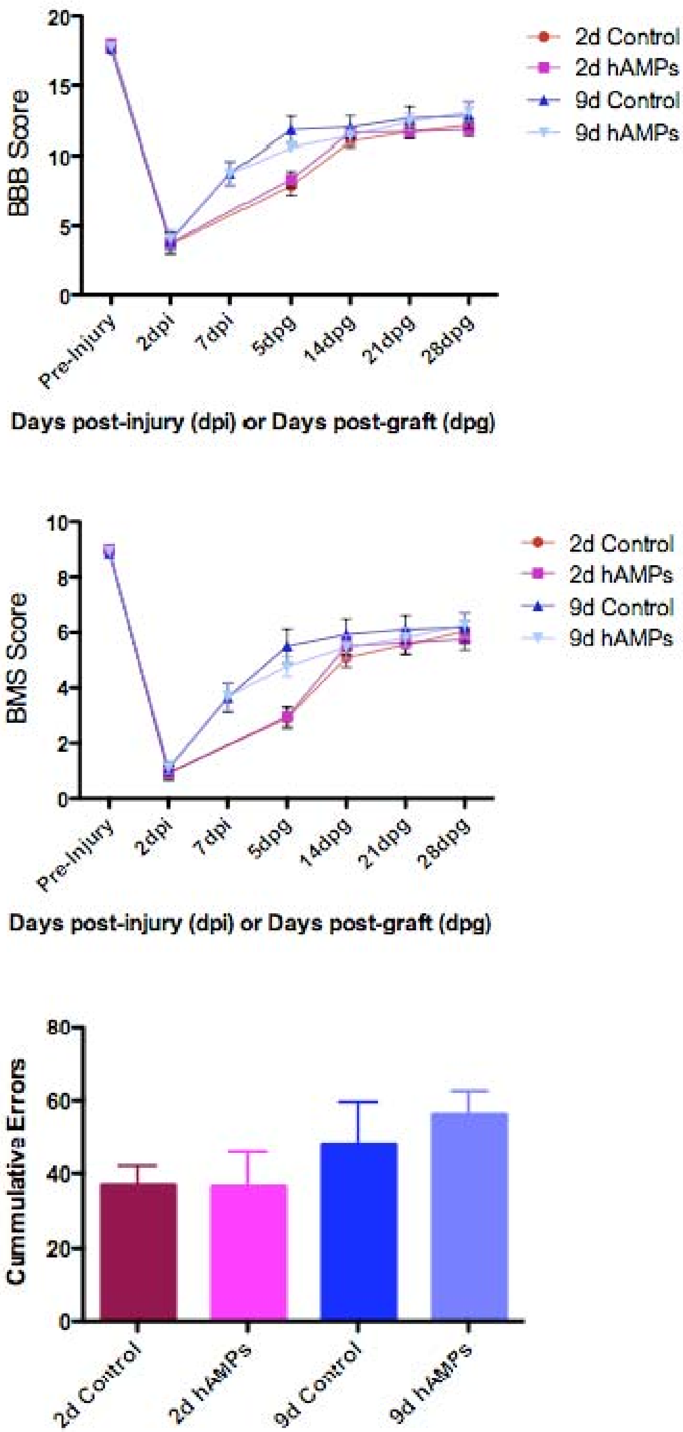


Figure 1. Open Field Locomotor and Horizontal Ladder Beam Assessments for Project 51.3. BBB (A) and BMS (B) scores for 2d and 9d post-SCI experimental groups. No statistically significant differences were found between groups in repeated measures ANOVA analysis with post-hoc Bonferoni Dunn correction. C) Supplemental behavioral analysis of horizontal ladder beam testing focusing on errors in paw placement during crossing revealed no statistically significant differences between animals that received hAMP transplants versus vehicle controls for either 2d or 9d post-SCI groups.